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Proteomic Analysis of Plasma From Patients During an Acute Coronary Syndrome

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OBJECTIVES The aim of this study was to analyze modifications in the plasma protein map during an acute coronary syndrome (ACS) using proteomics.

BACKGROUND Proteomics is a new technology that allows the detection and identification of several proteins at a given time in a sample.

METHODS Plasma from 19 patients, 11 with acute myocardial infarction (AMI) and 8 with unstable angina (UA), was investigated. The control group included nine age-matched volunteers.

RESULTS In two-dimensional electrophoresis using a pH range of 4 to 7, constant differences were found in at least four different areas within the plasma protein map. In area 1, we identified the presence of seven alpha$_1$-antitrypsin (AAT) isoforms in plasma from control subjects. alpha$_1$-antitrypsin isoform 1 was undetectable in plasma from UA and AMI patients. The AAT isoforms 5, 6, and 7 were reduced in plasma from AMI patients when compared with UA patients. Three fibrinogen gamma chain isoforms were identified in area 2. Fibrinogen gamma chain isoforms 1 and 2 were increased in AMI patients with respect to UA patients. Five apolipoprotein A-I isoforms were identified in area 3. All of them were reduced in plasma from AMI patients with respect to UA patients. In area 4, the γ-immunoglobulin heavy chains were detected and were found increased in plasma from ACS patients.

CONCLUSIONS Plasma proteomic analysis makes it possible to develop a map of the protein isoforms that are expressed in plasma during an ACS. (J Am Coll Cardiol 2004;44:1578–83) © 2004 by the American College of Cardiology Foundation

Several studies have demonstrated changes in the plasma levels of biomarkers related to inflammation, platelet activation, coagulation, myocyte necrosis, and plaque rupture in patients with acute coronary syndromes (ACS) (1–3). Most of these plasma biomarkers are associated with clinical prognosis (4,5). Until now the investigators had to analyze each biomarker one by one. However, a technology termed proteomics has been recently developed to obtain a map of the proteins expressed in tissues, cells, or fluids, including plasma (6). Proteomics is the study of the proteome. The plasma proteome consists of all the proteins present at a given time in it, including the variety of modified proteins arising from alternative splicing of transcripts and/or from post-translational processing, resulting in modifications that can alter protein structure and/or function.

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Proteomics uses a combination of techniques, including two-dimensional gel electrophoresis, image analysis, and mass spectrometry (7). This technique has been extensively employed to investigate cancer and other diseases (8,9), but there are currently no reports concerning the proteomic study of plasma from patients during ACS. In this study, we used proteomics to analyze modifications in the plasma protein map during unstable angina (UA) and acute myocardial infarction (AMI).

METHODS

Patients. Nineteen patients admitted to the Emergency Unit of Fundación Jiménez Díaz were investigated. Eleven had transmural AMI based on prolonged chest pain, changes on standard electrocardiogram, and raised serum creatine kinase (>2-fold upper limit of normal value of the biochemical laboratory) and creatine kinase, MB fraction (>10% total creatine kinase). Eight patients had UA based on chest pain at rest and transient S-T segment changes, without significant increases in creatine kinase and creatine kinase, MB fraction. The control group included nine age-matched volunteers.

The criterion for enrollment was admission within 24 h after the onset of chest pain. A criterion for exclusion was a previous episode of ACS. Because this work is included within a study to analyze molecular mechanisms of anti-

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Abbreviations and Acronyms

AAT	= alpha$_1$-antitrypsin
ACS	= acute coronary syndrome
AMI	= acute myocardial infarction
Apo A-I	= apolipoprotein A-I
2-DE	= bidimensional electrophoresis
MALDI-TOF MS	= matrix-assisted laser desorption/ ionization-time of flight mass spectrometry
MS/MS	= tandem mass spectrometry
UA	= unstable angina

thrombotic drugs, an exclusion criterion was also recent exposure (during the preceding two weeks) to antithrombotic, steroidal and nonsteroidal anti-inflammatory drugs, and heparin. Moreover, patients or control subjects with a history of neoplastic, infectious, or autoimmune diseases or any surgical procedure in the preceding six months were not included. All subjects gave informed consent, and the study was approved by the ethics committee of Fundación Jiménez Díaz.

Two-dimensional electrophoresis (2-DE). For 2-DE, 250 μg of plasma were diluted in 350 μl of 8 mol/L urea, 2% CHAPS w/v, 40 mmol/l dithiothreitol, 0.2 % Bio-Lyte ampholyte (Bio-Rad Labs, Hercules, California), and 0.01% w/v bromophenol blue. The samples were loaded on immobilized pH gradient gel strips (pH 3 to 10 or pH 4 to 7), and isoelectric focusing was performed using a Protean IEF cell system (Bio-Rad Labs). The gels were actively rehydrated at 50 V for 60 h, then rapid and linear voltage ramping steps, limited by a maximum current of 50 μA per gel, were applied at 250 V, 10,000 V, 10,000 V (30,000 Vh). In the second dimension, the proteins from the strips were resolved on 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis gels using a Protean II XL system (Bio-Rad Labs). Afterwards, the gels were fixed and silver stained. At least two different gels were run per patient.

Silver staining. The gels were fixed in a solution containing a Fixative Enhance Concentrate solution (Bio-Rad Labs), methanol, and acetic acid in distilled water for 20 min. After two 10-min washings in distilled water, gels were stained using a Silver Stain Plus Kit. Development was performed for approximately 25 min and stopped with 5% acetic acid. The stained gels were then washed twice in distilled water for 5 min each time.

Image acquisition and analysis. The stained gels were scanned in a UMAX POWERLOOK III Scanner operated by the software Magic Scan V 4.5. Intensity calibration was carried out using an intensity stepwedge before gel image capture. Image analysis was carried out using PD Quest 6.2.1 and Quantity One 4.2.3 (Bio-Rad Labs). Image spots were initially detected, matched, and then manually edited. Each spot intensity volume was processed by background subtraction, and total spot volume was normalized by the corresponding spot volume of albumin.

Mass spectrometry. The spots of interest were manually excised from the gels using biopsy punches. To identify the spots of interest by mass spectrometry, five different subjects from each experimental group were chosen. For each subject, the spots of interest were isolated from two different gels. Proteins selected for analysis were in-gel reduced, alkylated, and digested with trypsin. Briefly, the spots were washed twice with water, shrunk with 100% acetonitrile, and dried in a Savant SpeedVac. Afterwards, the samples were reduced with dithioerytritol in ammonium bicarbonate and subsequently alkylated with iodoacetamide in ammonium bicarbonate. Finally, the samples were digested with 12.5 ng/μl sequencing grade trypsin (Roche Molecular Biochemicals, Barcelona, Spain) in 25 mM ammonium bicarbonate (pH 8.5). After digestion, the supernatant was collected and 1 μl was spotted onto a matrix-assisted laser desorption/ionization (MALDI)

Table 1. Characteristics of the Groups Studied

	Age-Matched Control Group (n = 9)	Unstable Angina Group (n = 8)	Acute Myocardial Infarction Group (n = 11)
Age (yrs) (±SEM)	60.78 ± 2.4	76.8 ± 2.5	62.3 ± 3.4
Male/female	6/3	5/3	6/5
Risk factors, n (%)			
Tobacco smoking	2 (22%)	2 (25.0%)	4 (36.4%)
Hypertension	5 (55.5%)	4 (50.0%)	3 (27.3%)
Hypercholesterolemia (total cholesterol >200 mg/dl)	0 (0%)	1 (12.5%)	6 (54.5%)
Diabetes mellitus	2 (22%)	2 (25.0%)	1 (9.1%)
Medication on admission, n (%)			
Beta-blockers	0 (0%)	0 (0%)	0 (0%)
Calcium antagonists	1 (11%)	5 (62.5%)	1 (9.1%)
ACE inhibitors	0 (0%)	1 (12.5%)	1 (9.1%)
Diuretics	0 (0%)	2 (25.0%)	1 (9.1%)
Nitrates	0 (0%)	7 (87.5%)	11 (100%)
Statins	0 (0%)	1 (12.5%)	1 (9.1%)

ACE = angiotensin-converting enzyme.

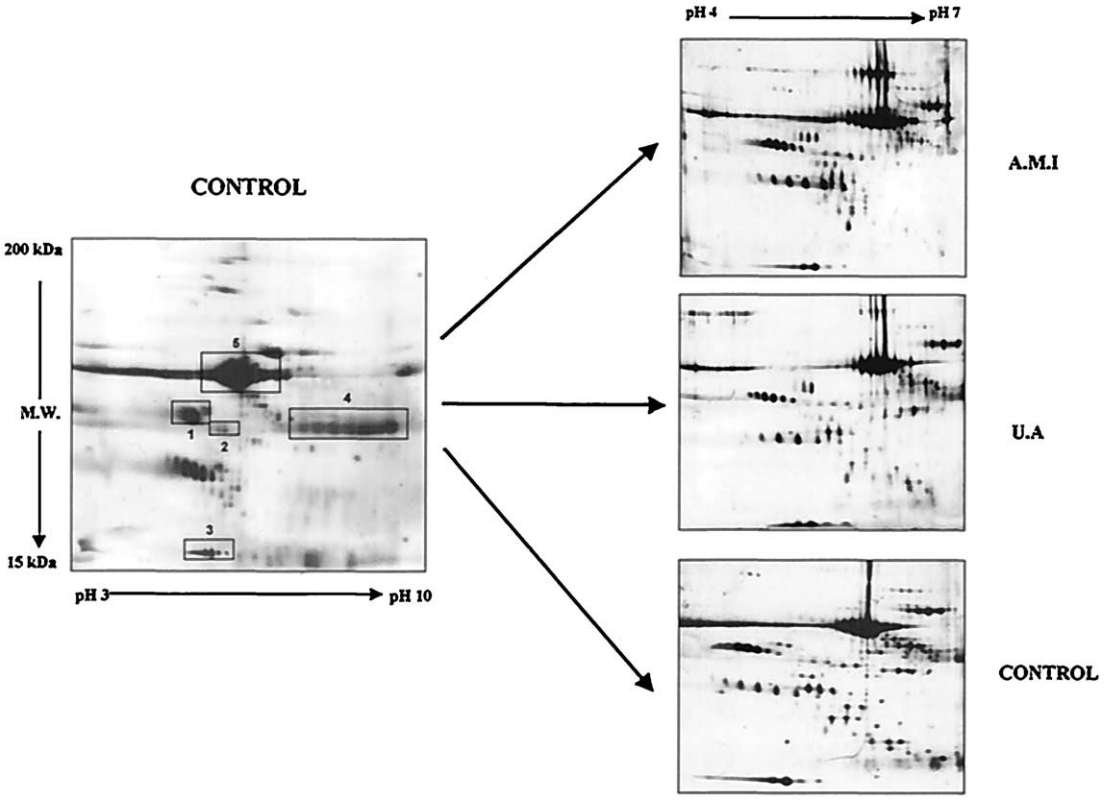

Figure 1. (Left) Two-dimensional gel of plasma from a control volunteer using a pH range of 3 to 10. Four areas were analyzed **(1 to 4)** in each group of subjects. **(Right)** Representative bidimensional electrophoresis gel images of plasma from patients during acute myocardial infarction (AMI), unstable angina (UA), and age-matched control subjects using a pH range of 4 to 7. Albumin was identified in area 5 and was used to normalize total spot volume in each of the other areas.

target plate. The MALDI-time of flight mass spectrometry (MALDI-TOF MS) analyses were performed in a Voyager-DE STR instrument (PerSeptives Biosystems, Framingham, Massachusetts), a model fitted with a 337-nm nitrogen laser and operated in reflector mode, with an accelerating voltage of 20,000 V. All mass spectra were calibrated externally using a standard peptide mixture (Sigma-Aldrich, Madrid, Spain). Peptides from the autodigestion of trypsin were used for internal calibration. The analysis by MALDI-TOF MS produces peptide mass fingerprints, and the peptides observed can be collated and represented as a list of monoisotopic molecular weights. Tandem mass spectrometry (MS/MS) was performed when: 1) the individual ion scores of the peptide mass fingerprints (score is −10 Log [P] where P is the probability that the observed match is a random event) did not reach identity or extensive homology with proteins contained in the Mascot database (10) or 2) peptides with similar ions scores matched different proteins. In some cases, MS/MS was carried out using the MALDI-tandem time-of-flight mass spectrometer 4700 Proteomics Analyzer (Applied Biosystems, Framingham, Massachusetts). The MS was performed by the Genomics and Proteomics Unit of Universidad Complutense, Madrid, Spain.

Statistical methods. Results are expressed as means ± SEM. To determine statistical significance, we performed the Mann-Whitney test. A p value <0.05 was considered statistically significant.

RESULTS

Protein separation. Table 1 shows the clinical features of the patients. Figure 1 shows a representative two-

Table 2. Identification of the Analyzed Proteins

Protein	Peptides Matched	Confirmation Method	Sequence Coverage (MS)	Sequence Coverage (MS/MS)
Alpha-1-antitrypsin	3–10	MS, plasma map	13%–58%	—
Apolipoprotein A-I	17–55	MS, plasma map, MS/MS	22%–86%	4%
Fibrinogen gamma chain	8–10	MS, plasma map, MS/MS	16%–28%	3%
Inmunoglobulin gamma heavy chain	—	Plasma map	—	—
Albumin	—	Plasma map	—	—

MS: mass spectrometry; MALDI-TOF MS analyses (10); Plasma map: SWISS-PROT 2D database (11); MS/MS: Tandem mass spectrometry (12).
MALDI-TOF MS = matrix-assisted laser desorption/ionization time of flight mass spectrometry.

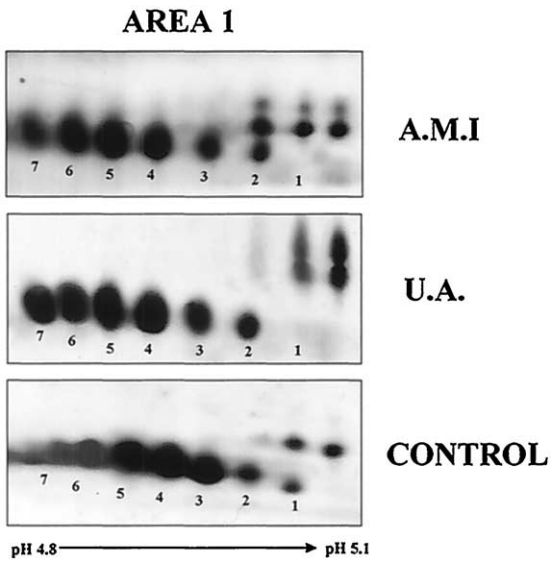

Figure 2. Representative pattern of alpha$_1$-antitrypsin (AAT-1) in plasma from patients during acute myocardial infarction (AMI), unstable angina (UA), and age-matched control subjects. Images were obtained from the bidimensional electrophoresis gels using a pH range of 4 to 7. **Numbers 1 to 7** were only used to identify each AAT isoform.

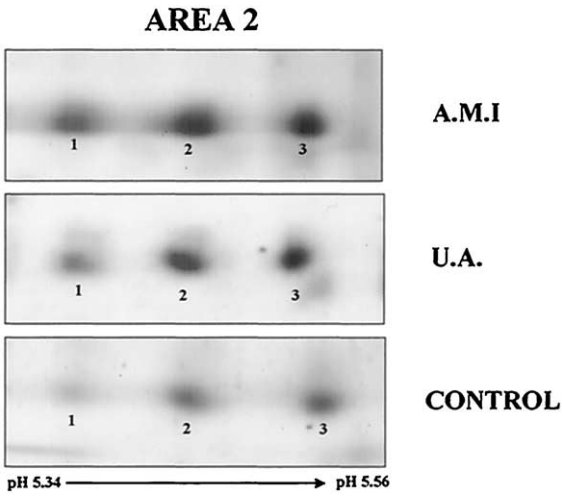

Figure 3. Representative pattern of fibrinogen gamma chain in plasma from patients during acute myocardial infarction (AMI), unstable angina (UA), and age-matched control subjects. Images were obtained from the bidimensional electrophoresis gels using a pH range of 4 to 7. **Numbers 1 to 3** were only used to identify each fibrinogen gamma chain isoform.

dimensional gel image of control plasma. More than 400 spots were detected ranging from 15 to 200 kDa with a pH between 3 and 10. Four different areas localized within a pH range of 4 to 7 in the proteomic plasma map (1 to 4) were observed showing constant differences between the different experimental groups (Fig. 1, left). Two-dimensional amplifying gels with a pH range of 4 to 7 were run (Fig. 1, right).

Protein identification. The most remarkable spots whose density had changed in these areas were densitometrically measured and identified by comparison with those in the SWISS-2D database plasma map (11) (Table 2). Their identity was confirmed by mass spectrometry (Table 2). For this purpose, they were subjected to MALDI-TOF MS and database searching (12). When required, MS/MS was further performed (Table 2). Densitometric analysis of each spot was calculated with albumin as reference (detected in area 5).

Alpha$_1$-antitrypsin (AAT) was identified in area 1. In control subjects, we detected seven different isoforms numbered 1 to 7 (Fig. 2). In both UA and AMI, isoform 1 was undetectable (Fig. 2, Table 3). Isoforms 5, 6, and 7 in UA and isoforms 6 and 7 in AMI were increased with respect to control subjects (Table 3). Moreover, isoforms 5, 6, and 7 were significantly reduced in AMI when compared with UA (Table 3).

In area 2, we observed three isoforms of fibrinogen gamma chain (Fig. 3, Table 4). Isoforms 1 and 2 were significantly increased in ACS when compared with control subjects (Table 4) and were significantly increased in AMI when compared with UA (Table 4).

Five apolipoprotein A-I (Apo A-I) (area 3) isoforms were observed (Fig. 4). Four Apo A-I isoforms were significantly reduced in AMI when compared with control subjects (Table 5), and the five isoforms were significantly reduced in AMI when compared with UA (Table 5). Isoforms 2 and 3 were significantly increased in UA when compared with control subjects (Table 5).

In area 4, the immunoglobulin gamma heavy chains were detected and were significantly increased in ACS when compared with control subjects (Table 5).

Table 3. α-1-Antitrypsin Isoforms in Plasma From Patients During an Acute Coronary Syndrome

Protein	Map Area	Experimental Mass (kDa/pI)	Age-Matched Control Group (AU)	Unstable Angina Group (AU)	Acute Myocardial Infarction Group (AU)
AAT isoform 1	1	53.5/5.1	28.8 ± 1.08	U.D.	U.D.
AAT isoform 2	1	55.6/5.0	41.59 ± 4.38	49.0 ± 6.6	43.1 ± 5.2
AAT isoform 3	1	54.8/5.0	66.39 ± 5.4	59.8 ± 5.7	55.6 ± 5.5
AAT isoform 4	1	56.1/5.0	78.04 ± 1.96	78.5 ± 4.3	76.3 ± 2.7
AAT isoform 5	1	56.5/4.9	76.81 ± 1.68	88.2 ± 2.3*	80.4 ± 2.2†
AAT isoform 6	1	56.2/4.9	64.57 ± 3.26	88.1 ± 2.7*	77.2 ± 3.2*†
AAT isoform 7	1	56.1/4.9	44.75 ± 3.67	80.9 ± 3.4*	63.6 ± 4.5*†

*$p < 0.05$ with respect to age-matched control group. †$p < 0.05$ with respect to unstable angina patients. Densitometric analysis of the different AAT isoforms detected in plasma from healthy volunteers and patients during unstable angina and acute myocardial infarction. Results are represented as mean ± SEM.

AAT = α-1-antitrypsin; AU = arbitrary units; UD = undetectable.

Table 4. Fibrinogen Gamma Chain Isoforms in Plasma From Patients During an Acute Coronary Syndrome

Protein	Map Area	Experimental Mass (kDa/pI)	Age-Matched Volunteers (AU)	Unstable Angina Group (AU)	Acute Myocardial Infarction Group (AU)
FGG isoform 1	2	49.5/5.3	16.9 ± 1.43	33.0 ± 2.4*	44.8 ± 4.0*†
FGG isoform 2	2	49.4/5.4	30.6 ± 2.8	38.4 ± 2.4*	51.2 ± 3.7*†
FGG isoform 3	2	49.4/5.6	32.4 ± 3.74	36.6 ± 3.6	42.2 ± 4.8

*$p < 0.05$ with respect to age-matched control group. †$p < 0.05$ with respect to unstable angina patients. Densitometric analysis of the different FGG isoforms observed in plasma from healthy volunteers and patients during unstable angina and acute myocardial infarction. Results are represented as mean ± SEM.
AU = arbitrary units; FGG = fibrinogen gamma chain.

DISCUSSION

In this study, we show for the first time in a single map the different proteins expressed in plasma from patients during an ACS, that is, UA and AMI, using proteomics. Proteomics is a new technology that allows the determination of the changes found in several proteins in a sample obtained at a given time, mainly by using 2-DE.

One of the main findings was that the different AAT isoforms change in plasma during an ACS. The AAT is a powerful inhibitor of several proteolytic enzymes, although neutrophil elastase is clearly its primary protease target (13). There is now ample evidence that, in the absence of enough AAT in the lower respiratory tract, there is an increased burden of proinflammatory factors (14). We found seven different isoforms in control subjects. The AAT isoform 1 was not present in ACS. There was a significant reduction of isoforms 5, 6, and 7 in AMI when compared with UA. As a theory, these data open the possibility that AMI may be associated with a failure to increase some AAT isoforms. In this line of evidence, it has been suggested that low AAT levels promote atherogenesis, and some AAT genotypes have been associated with a reduced risk of ischemic cerebrovascular and heart diseases (15,16). Moreover, AAT can inhibit neutrophil superoxide production, and exogenous administration of AAT conferred protection against ischemic/reperfusion injury (17,18). Another possibility is that during myocardial infarction significant quantities of these AAT isoforms may be consumed. Further characterization of the function of each AAT isoform may reveal in-depth information about the mechanisms of ACS.

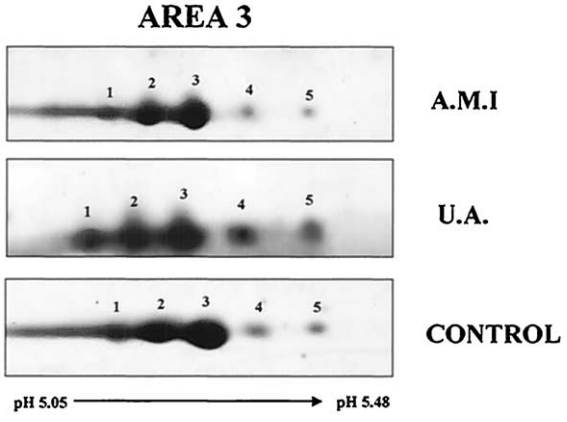

Figure 4. Representative patterns of apolipoprotein A-I in plasma from patients during acute myocardial infarction (AMI), unstable angina (UA), and age-matched control subjects. Images were obtained from the bidimensional electrophoresis gels using a pH range of 4 to 7. **Numbers 1 to 5** were only used to identify each apolipoprotein A-I isoform.

Fibrinogen is essential for fibrin formation under the influence of thrombin and forms the basic plug for plasma coagulation and platelet aggregation via glycoprotein IIb/IIIa receptors (19). Furthermore, fibrinogen increases the binding of platelets, endothelial cells, and leukocytes to each other, which in turn causes leukocyte and platelet activation and release of mediators from these cells (20).

Fibrinogen contains two sets of three chains (alpha, beta, and gamma) linked by disulfide bonds (21). Activated platelets bind to fibrin via the N-terminal of the beta-chain and the extreme C-terminal of the gamma chain (22). Marshall et al. (23), using mass spectral fingerprinting, have identified fibrinogen fragments in the serum of patients during myocardial infarction. In our study we detected three different fibrinogen gamma chain isoforms in plasma from normal and ACS patients. Two of them increased in AMI compared with UA. Interestingly, a different alternative splicing of fibrinogen gamma chain messenger ribonucleic acid has been recently demonstrated that results in fibrinogen gamma chain variants that alter fibrin formation and structure and could be associated with increased thrombotic risk (24).

Apolipoprotein A-I is a major component of high-density lipoproteins. An inverse relationship between Apo A-I levels and coronary heart disease has been suggested (25,26). Indeed, several beneficial cardiovascular effects of high-density lipoproteins have been attributed to Apo A-I (27). In our study, we detected five Apo A-I isoforms in control plasma and in ACS. However, the expression of the five isoforms was greater in UA than in AMI. As a hypothesis, the increased Apo A-I expression in UA compared with AMI could be theoretically associated with the severity of the coronary syndrome, UA versus AMI. Accordingly, increased Apo A-I during UA could imply an antagonistic response to the inflammatory reaction associated with the cardiac event. In fact, anti-inflammatory properties of Apo A-I have been reported (28).

We finally observed an increased level of immunoglobulin gamma heavy chains in ACS compared with control subjects. This finding could be related to the inflammatory character of ACS.

Proteomic analysis permits the detection of different

Table 5. Expression of Apolipoprotein A-I Isoforms and Gamma Immunoglobulin Heavy Chains in Plasma From Patients During an Acute Coronary Syndrome

Protein	Map Area	Experimental Mass (kDa/pI)	Age-Matched Volunteers (AU)	Unstable Angina Group (AU)	Acute Myocardial Infarction Group (AU)
Apo A-I isoform 1	3	23.1/5.0	39.7 ± 1.75	58.7 ± 10.4	17.0 ± 3.5*†
Apo A-I isoform 2	3	23.0/5.1	56 ± 1.09	74.9 ± 6.5*	47.5 ± 4.6†
Apo A-I isoform 3	3	23.1/5.2	67.9 ± 1.54	80.4 ± 3.2*	52.5 ± 3.1*†
Apo A-I isoform 4	3	23.3/5.4	20.3 ± 2.39	45.0 ± 7.9	5.2 ± 1.5*†
Apo A-I isoform 5	3	23.6/5.5	11.7 ± 1.34	37.8 ± 11.7	2.1 ± 1.1*†
Immunoglobulin gamma heavy chains	4	50.6–54.6/6.1–9.0	59.65 ± 2.3	69.5 ± 2.3*	65.0 ± 2.9*

*$p < 0.05$ with respect to age-matched control group. †$p < 0.05$ with respect to unstable angina patients. Densitometric analysis of the Apo A-I isoforms found in plasma from healthy volunteers and patients during an acute coronary syndrome. Results are represented as mean ± SEM.

Apo A-I = apolipoprotein A-I; AU = arbitrary units.

protein isoforms that are not identified by conventional measurement of the circulating biomarkers that could be useful to understand the molecular mechanisms involved in ACS. Our observations should be interpreted with caution. First, quantification using two-dimensional electrophoresis is not an exact method, because gel spots can contain multiple proteins and because mass spectrometry is not quantitative. Therefore, other methods such as immunoblotting should be used. Also, the number of patients is small and larger groups are needed to establish the clinical importance of these findings. The present work is merely an example of the use of proteomics to identify several protein isoforms at a given time in plasma from patients during an ACS.

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REFERENCES

1. Oldgren J, Wallantin L, Grip L, Linder R, Norgaard BL, Siegbahn A. Myocardial damage, inflammation and thrombin inhibition in unstable coronary artery disease. Eur Heart J 2003;24:86–93.
2. Newby LK, Goldmann BU, Ohman EM. Troponin: an important prognostic marker and risk-stratification tool in non–ST-segment elevation acute coronary syndromes. J Am Coll Cardiol 2003;41 Suppl S:31S–6S.
3. Collet JP, Montalescot G, Vicant E, et al. Acute release of plasminogen activator inhibitor-1 in ST-segment elevation myocardial infarction predicts mortality. Circulation 2003;108:391–4.
4. Baldus S, Heeschen C, Meinertz T, et al. Myeloperoxidase serum levels predicts risk in patients with acute coronary syndromes. Circulation 2003;108:1440–5.
5. Varo N, de Lemos JA, Libby P, et al. Soluble CD40L: risk prediction after acute coronary syndromes. Circulation 2003;108:1049–52.
6. Parekh R. Proteomics and molecular medicine. Nat Biotechnol 1999; 17:19–20.
7. Chabers G, Lawrie L, Cash P, Murray GI. Proteomics: a new approach to the study of disease. J Pathol 2000;192:280–8.
8. Ahram M, Best CJ, Flaig MJ, et al. Proteomic analysis of human prostate cancer. Mol Carcinogenesis 2002;33:9–15.
9. Srinivas PR, Srivastava S, Hanash S, Wright GC. Proteomics in early detection of cancer. Clin Chem 2001;47:1901–11.
10. Mascot database. http://www.matrixscience.com. Accessed between September 2003 and March 2004.
11. SWISS-2D database. http://www.expasy.ch/ch2d/. Accessed between September 2003 and March 2004.
12. Mascot database. http://www.matrixscience.com. Accessed between September 2003 and March 2004.
13. Travis J, Salvesem GS. Human plasma proteinase inhibitors. Ann Rev Biochem 1983;52:655–709.
14. Hubbard RC, Brantly ML, Sellers SE, Mitchell ME, Crystal RG. Anti-neutrophil-elastase defenses of the lower respiratory tract in alpha 1-antitrypsin deficiency directly augmented with an aerosol of alpha 1 antitrypsin. Ann Intern Med 1989;111:206–12.
15. Tamud PJ, Martin S, Steiner G, et al. Progression of atherosclerosis is associated with variation in the alpha 1-antitrypsin gene. Arterioscler Thromb Vasc Biol 2003;23:644–9.
16. Dahl M, Tybjaerg-Hansen A, Sillesen H, Jensen G, Steffensen R, Nordestgaard BG. Blood pressure, risk of ischemic cerebrovascular and ischemic heart disease and longevity in alpha (1)-antitrypsin deficiency in the Copenhagen City Heart Study. Circulation 2003;107:747–52.
17. Bucurenci N, Blake DR, Chidwick K, Winyard PG. Inhibition of neutrophil superoxide production by human plasma alpha 1-antitrypsin. FEBS Lett 1992;300:21–4.
18. Daemen M, Heemskerk VH, Van't Veer C, et al. Functional protection by acute phase proteins α_1-acid glycoprotein and α_1-antitrypsin against ischemia/reperfusion injury by preventing apoptosis and inflammation. Circulation 2000;102:1420–6.
19. Mannucci PM. Recent progress in the pathophysiology of fibrinogen. Eur Heart J 1995;16:25–30.
20. Bombeli T, Schwartz BR, Harlam JM. Adhesion of activated platelets to endothelial cells: evidence for a GP IIb/IIIa dependent bridging mechanism and novel roles for endothelial intercellular adhesion molecule 1 (ICAM-1), alphavbeta3, integrin and GP Ib alpha. J Exp Med 1998;187:329–39.
21. Everse SJ, Spraggon RF, Doolittle RF. A three dimensional consideration of variant human fibrinogens. Thromb Haemost 1998;80:1–9.
22. Farrell DH, Thiagarajan P, Chung DW, Darie EW. Role of fibrinogen α and γ chain sites in platelet aggregation. Proc Natl Acad Sci USA 1992;89:10729–32.
23. Marshall J, Kupchak P, Zhu W, Yantha J, Vrees T, Furesz S. Processing of serum proteins underlies the mass spectral fingerprinting of myocardial infarction. J Proteome Res 2003;2:361–72.
24. Cooper AV, Standeven KF, Ariens RA. Fibrinogen gamma-chain splice variant gamma alters fibrin formation and structure. Blood 2003;102:535–40.
25. Puchois P, Kandoussi A, Fievet P, et al. Apolipoprotein A-I containing lipoproteins in coronary artery disease. Atherosclerosis 1987;68: 35–40.
26. Duverger N, Kruth H, Emmanuel F, et al. Inhibition of atherosclerosis development in cholesterol-fed human Apolipoprotein A-I transgenic rabbits. Circulation 1996;94:713–7.
27. Rämet ME, Rämet M, Lu Q, et al. High-density lipoprotein increases the abundance of eNOS protein in human vascular endothelial cells by increasing its half-life. J Am Coll Cardiol 2003;41:2288–97.
28. Dimayuga P, Zhu J, Oguchi S, et al. Reconstituted HDL containing human Apolipoprotein A-I reduces VCAM-1 expression and neointima formation following periadventitial cuff-induced carotid injury in Apo E null mice. Biochem Biophys Res Commun 1999;264:465–8.